SARS-CoV-2 intra-host evolution during acute infection in COVID-19 patients

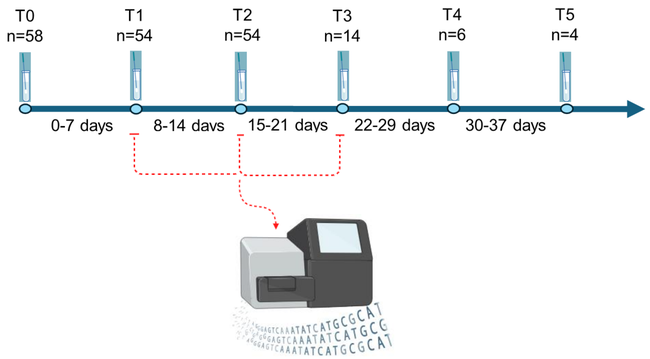
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BACKGROUND

Intra-host evolution represents the mechanism behind the continued emergence of new, highly divergent SARS-CoV-2 variants, the monitoring of which, through whole-genome sequencing, is critical to track the emergence of viral mutations. It has been speculated that the emergence of antigenically diverse SARS-CoV-2 variants results from intra-host viral evolution driven initially by suboptimal immune responses and then spread by forward transmissions. Persistent infection, even in not dramatically immunocompromised patients, leads to continued local emergence of SARS-CoV-2 variants if there is high-level community transmission.

METHODS

58 cases of COVID-19 with 2 or more longitudinally positive SARS-CoV-2 swabs collected at intervals of at least 7 days from the first positive swab were analysed, as shown in the graphic below. Study was conducted at Luigi Sacco Hospital in Milan in the period 2021-2023.

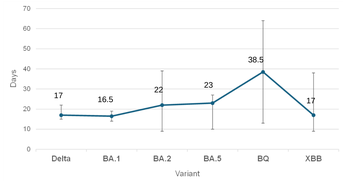


All samples were analyzed by Real Time PCR for cycle threshold (Ct) determination. Only samples with ct ≤ 32 were sequenced by Next Generation Sequencing (Miseq Illumina, 2 × 200 cycle paired-end). Samples with high coverage after sequencing were used for minority mutation analysis.
The alignment, clade assignment and mutation calling were conducted by Bioedit program and Nextstrain.
Statistical analyses included parametric (Pearson $\chi 2$ or Fisher exact test) and non-parametric tests (Wilcoxon Mann-Whitney and Kruskal Wallis Test).

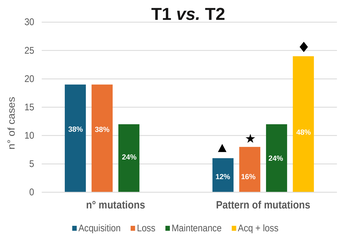
There is a **multiple steps** selection mechanism for the fitness of SARS-CoV-2 mutations. It is a *combination* of **selective pressure** and **diverse viral population**, independently of viral variant

RESULTS

The median age of subjects was 83 years (IQR:73-88), and 55.2% (n=32) were males. 57/58 subjects were hospitalized for a median of 23 days(IQR:16-32) and 12 died (21%) after a median of 25 days of hospitalization (IQR:19-34). None had a documented previous SARS-CoV-2 infection, 79% (n=45) were vaccinated and 46.7% (n=21) had received 3 doses. 7 subjects had no comorbidities and therefore received no treatment, while 22.5% had received more than one treatment for SARS-CoV-2 infection (48% had received tixagevimab/cilgavimab + nirmatrelvir/ritonavir). 60% of the subjects with monotherapy had received Remdesivir.

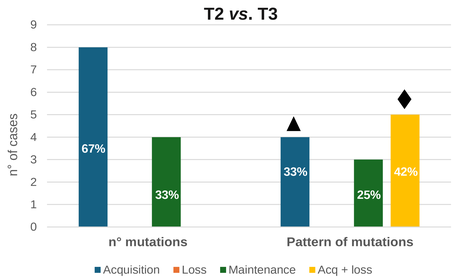


Several viral variants were observed, 24% (n=14) carried BA.2 and 31% were XBB.
Negativization period showed longer intervals in BQ.1 (39 days) and shorter in BA.1 infections (17 days, *p=.04*).



No drug resistance mutations were observed over time.
Comparing T1 *vs*. T2, 38% decreased/increased number of mutations and 24% maintained the same number. Subjects with constant numbers of mutations maintained the same pattern while 50% of the acquisitions and losses were confirmed (▲★). Acquired mutations at T2 were not present as minority mutations at T1, 50% of the lost mutations did not persist at T2 (♦).

In T2 *vs*. T3, 67% and 33% increased or maintained the same number of mutations, respectively. 75% of subjects with constant number of mutations maintained the same pattern, 50% of the acquisitions were confirmed (▲). 42% of the subjects had a combination of acquisition and loss of mutations (♦). New mutations at T3 were not present as minority mutations at T2.



Subjects with cardiovascular disease showed a significant acquisition of mutations over time (*p=.048*).

CONCLUSIONS

Our data highlight the presence of multiple steps in the fitness selection of SARS-CoV-2 mutations:

- ✓in the first step/week randomized mutations are generated indicating a genetic diversity increase
- ✓in the second step/week a purifying selection process was observed, determining a reduction in the mutations number
- ✓in the third step/week a further increase of novel mutations was observed in subjects with prolonged infection.

The fixation of a few mutations, and the appearance of others not previously detected, suggests a combination of selective pressures and a diverse viral population independently of the viral variant considered.
These results suggested that around 50% of all COVID-19 patients develop highly divergent variants within 3 weeks of infection regardless of treatment.

ADDITIONAL KEY INFORMATION

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